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Cell Membrane Coated Particles

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Abstract
Nanoformulations are widely considered in the biomedical field for drug delivery, imaging or detoxification purposes. Cell membrane coatings are a growing concept that aims to camouflage nanomaterials. The cell membranes are the first point of contact for cells to other biological or synthetic materials and nature has established signaling pathways in this context. In contrast to using purified membrane associated proteins, the use of purified cell membranes contains the protein of interest in a very native environment. This Progress Report provides an overview over the advances in cell membrane coated (nano)particles from the past 2-3 years. The progress in using cell membranes from mammalian cells without nuclei i.e., red blood cells and platelets, as well as nucleus-containing cells in particular white blood cell and specific cancer cells is outlined. Additionally, highlight from recent reports considering hybrid cell membrane coating that originate from at least two different cell types are discussed Finally, a future perspective indicating the challenges and potential of cell membrane coated nanomaterials and biomaterials is provided.

1. Introduction
Nanoparticle (NP)-based formulations are being explored over the past decades with aim to improve the therapeutic efficacy of therapeutic cargo. The types of NPs used are very diverse including polymer-based micelles and vesicles, inorganic and metal NPs or lipid-based assemblies. Similarly, the strategies to equip these formulations with ability to hide from the immune systems and/or targeting to the diseased sites are diverse reaching from simple conjugation with poly(ethylene glycol) (PEG) to multi-step modifications with different entities aiming for theranostic applications. These different nanoformulations often rely on complex chemistry and purified single biomolecules that can be controlled in simple buffer environments, but that potentially face multifaceted challenges in complex biological environments including stability issue, undesired degradation, triggered immune response and systemic removal before reaching the target among others, resulting in underperforming therapeutic outcomes even in simple mouse models.[1]

From a different perspective, multiple cell types in a human body can move unhindered towards a target. It is remarkable that red blood cells (RBCs) circulate over 100 days although they are larger than the smallest capillaries. Nature not only employs the surface chemistry of the cells, but also their geometry and mechanical properties, i.e., the biconcave shape of the RBCs, give them the deformability to pass capillaries. Therefore, efforts to mimic RBCs are being reported from fabricating geometrically matching entities to matching their chemical composition.[2] However, making an artificial copy of a cell is challenging. Hence, the compromise between relying on a single interaction point (typically receptor-antibody interactions) of nanoformulations with cells and tissue, and the great complexity of an entire cell is currently a more realistic approach. An interesting example in this context is the use of purified cell membrane to camouflage (nano)particles. This concept offers opportunity to mimic cell-cell interactions employing multiple types of contact points while avoiding the need to obtain/purify
specific proteins. This nature-inspired approach was first shown in 2011 with RBC membranes to enhance particle blood circulation times and developed in the next 4-5 years to include white blood cells, platelets and cancerous cell sources. The field gained increasing attention over the past years, expanding on types of cell sources, coated particles and envisioned applications. This progress report will highlight selected efforts in the field from the past 2-3 years focusing on mammalian cell sources and coating of colloidal systems (Scheme 1). Efforts pre-dating 2017/2018 were already discussed in detail in several reviews. First, a short overview over the general cell membrane purification procedure and subsequent characterization will be provided. Then, we will outline advances made when non-nuclei or nuclei containing cells sources were used. Finally, coatings consisting of two types of cell membrane (hybrid coatings) will be discussed.

Scheme 1. a) Cell membrane coated particles are often typically obtained by purifying cell membranes from either cells in blood or cell culture followed by the vesicles formation and nanomaterial coating or direct coating of the nanoparticles. b) The cell membrane coated particles can have prolonged blood circulation times and/or improved targeting abilities, resulting in more efficient therapeutic outcome or disease site imaging (c).

2. Formation and characterization of cell membrane coated particles
Despite of the variation in cell sources, the general purification methods of cell membranes and sequential coating of particles are rather similar and therefore described only briefly. Furthermore, important characterization techniques for size, protein content and surface charge are discussed. We would like to note that there are a variety of other techniques not mentioned here specifically that can provide valuable specific information such as Fourier transform infrared spectroscopy for analysis of functional groups or energy dispersive X-ray spectroscopy for element recognition.

2.1. Particles coated with cell membranes
The initial step of getting access to purified cell membranes is the collection of the source cells. Culture cells, are typically detached from culture flasks or culture plates via scraping or
trypsinization followed by sequential centrifugation cycles, whereas cells from blood are normally collected directly via centrifugation. A variety of methods are utilized to break the cell membrane including hypnotic lysing, freeze-thaw cycles or mechanical force and combinations thereof followed by centrifugation cycles to remove cellular debris. It should be noted that ultracentrifugation is required and that cell membrane collection of cells lacking internal organelles is simpler compared to cells with nuclei due to additional separation steps. The obtained cell membranes can be directly used for coating via co-extrusion or sonication with the core particles. However, it is more common to first form cell membrane vesicles, before co-extrusion or sonication with the core particles. In addition to sonication and co-extrusion, coating of core particles with cell membranes using microfluidics electroporation was done, which showed improved colloidal stability compared to a co-extruded counterpart.[6] Based on the sources used in this progress report, the most common core particles are solid spherical anionic NPs with diameters between 50 nm and 200 nm. However, particles with sizes up to the low-micrometer range (< 10 μm) and with various shapes (rod, cubic and helical) as well as soft particles like nanosponges were coated with cell membranes.[7] Despite the majority of the core particles being anionic, cationic particles were successfully used as well, regardless of the potential issues with aggregation when mixed with negatively charged cell membranes.[8]

2.2. Characterization of cell membrane coated particles
Several techniques are frequently used to characterize the cell membrane coated particles (CM-P). Imaging via transmission electron microscopy can give insight in size and morphology of the CM-P. Importantly, the lipid bilayer can often be visualized as an indication of successful coating. Fluorescent microscopy is a powerful tool to show co-localization and successful coating of fluorescently labeled cores and lipid bilayers. In addition, fluorescent imaging can be used for confirmation of the presence of multiple types of cell membranes or incorporated artificial components. On the other hand, dynamic light scattering (DLS) is a quick tool to gain insight in size and size distributions of assembled CM-P, while zeta-potential measurements are employed to demonstrate the close resemblance of the CM-Ps’ surface charge with native cell membrane vesicles. The retention of proteins in the cell membranes after coating is essential. Protein quantification (e.g., the bicinchoninic acid assay or the Bradford protein assay) and/or sodium dodecyl sulfate-page polyacrylamide gel electrophoresis are normally used to gain insight in the quantity and type of proteins, respectively. Western blotting with appropriate antibodies is generally used to check for specific proteins that can be compared with the collected cell membranes or cell membrane vesicles to assess the protein retention during fabrication.

3. Non-nuclei containing cells sources
Cells without nuclei as sources for cell membranes are widely used. The collection of their membranes is rather straightforward due to the lesser amount of cellular debris compared to when nuclei-containing cell sources are used. The two types of non-nuclei containing cells predominantly used are RBCs and platelets. Both RBCs and platelets originate from blood and can equip particles with distinct properties. While coating of particles with RBC membranes increases blood circulation times, coating with platelet membranes has the advantage of targeting for inflammatory and tumor sites, which can be leveraged for drug delivery to these sites. However, the use of RBC membranes does not result in any specific targeting, while coating with platelets membranes only provides less improved blood circulation times compared to RBC membranes. The reader is referred to several reviews for a more detailed overview over this aspect.[9]
In a recent effort, both RBC and platelet membranes were successfully used to coat fullerene NPs (RFNP and PFNP, respectively) to treat thrombosis.[10] Results from rat studies showed that the closure of thrombosis sites was reduced when using RFNP (34.26%), which was slightly better than therapeutic urokinase (45.5%) while PFNP (68.42%) performed worse. The lower affinity of PFNP for thrombosis sites was indicated as the reason for these results. Furthermore, biosafety assays via tail bleeding showed that both RFNP (248 s) and PFNP (130 s) outperformed urokinase (383 s). Particles coated with RBC membranes retain the cluster of differentiation 47 (CD47) protein, an important “do not eat me” marker, responsible for prolonged blood retention times. In another effort, poly(lactic-co-glycolic acid) (PLGA) NPs were coated with RBC membranes for the removal of four different pore forming toxins (melittin, α-hemolysin, listeriolysin O and streptolysin O) from blood.[11] The effective concentration of NPs needed for hemolysis reduction of RBCs varied from 30 μg mL⁻¹ for listeriolysin O to 200 μg mL⁻¹ for α-hemolysin. In addition, 100% survival was seen for melittin, listeriolysin O and streptolysin O, but only 67% survival was determined for listeriolysin O when the previously mentioned concentrations of the pore forming toxins were used in mice. In another example, extended blood retention times due to a RBC membrane coating were utilized for tumor targeting via the enhanced permeability effect with the aim to provide a combined chemo and photodynamic therapy.[12] The designed CM-P could be light activated after endocytosis to create reactive oxygen species. Results from tumor growth reduction in mice showed that the tumor weight after treatment was <10 mg for the CM-P outperforming uncoated NPs (10 mg), while the control showed significant tumor growth (160 mg). Additionally, CM-P had reduced accumulation in the liver, which is beneficial to reduce side effects often related to chemotherapy. In a different effort, RBC membrane coated gold nanorods were made with the aim to use them in photothermal cancer therapy that uses near infrared (NIR) radiation to create heat and induce cell death.[7b] The RBC coated gold nanorods and the uncoated nanorods showed a 80% and 20% cell viability, respectively, in A549 cells when using a nanorods concentration of 100 μg ml⁻¹. Furthermore, the photothermal effect remained after coating the gold nanorods with RBC membranes, illustrated 10-fold higher A549 cell death after NIR irradiation for 8 min at 1.5 W cm⁻² compared to the control. Another example considered the use of stem cell factors in PLGA NPs that were coated with RBC membranes as a potential intervention for liver failure.[13] Fluorescence analysis of the tissue 6, 12 and 24 h after NPs injection showed a higher signal in the liver at all time-points. Results from in vivo test on mice showed increased survival (60%) with RBC membrane coated NPs compared to PBS control (10%) and uncoated NPs (30%).

The larger size of microparticles allows for higher loading efficacy but complicates passing small capillaries, and macrophage recognition reduces their blood circulation times. The fabrication of RBC-like microparticles (RBC-MP) with similar folding properties than the natural role models are circumventing these challenges, as mentioned above.[14] Hayashi et al modified microparticles with RBC membranes to create mimics (RBC-MP@RBCM) with had RBC-like shape, mechanical properties and surface coating (Figure 1aI).[15] RBC-MP@RBCM had a 4× higher blood retention compared to the uncoated controls after 24 h in mice (Figure 1aII). In another effort, bacteria were coated with RBC membranes (CMCB) and the potential of CMCB to avoid immune response and clearance was assessed.[16] The blood retention of CMCB was 14× higher compared to uncoated bacteria after 48 h in mice using colony forming units. Moreover, inflammatory markers indicated a lower immune response towards CMCB than bare bacteria. Notably, the RBC membrane coating was lost upon bacteria cell division. Further, RBC membrane coatings can also play a valuable role in coating imaging agents to improve their blood circulation times. For instance, Park and co-workers coated hollow mesoporous NPs with RBC membranes.[17] The mice used for the in vivo studies were also utilized as source for the RBC membranes with the aim to minimize immunogenic response towards the NPs. The NPs were equipped with ⁸⁹Zr as imaging agent because the long lasting
signal from $^{89}$Zr should complement with the long blood circulation times of the RBC membrane coated NPs. Positron-emission tomography (PET) imaging in mice showed that the signal from RBC membrane coated NPs was found throughout the body, while uncoated NPs accumulated extensively in liver and spleen after 7 days. In a different effort, upconverting NPs (NaGdF$_4$:Yb,Tm) coated with RBC membranes and an incorporated linker for click chemistry were accumulated in a tumor site in a mouse model.[18] Then, modified PET agent $^{18}$F were injected and attached to the pre-accumulated NPs via click chemistry. A PET signal from the tumor site was detectable after 30 min, indicating the feasibility of the approach. In an alternative effort, gadolinium (Gd), another magnetic resonance imaging (MRI) agent, suffers from quick removal from the bloodstream, limiting its clinical effectiveness. Therefore, Gd was attached to a PLGA NP core and coated with RBC membranes (RBC-Gd-PLGA NP) to enhance blood circulation times.[19] The circulation time in blood was determined in mice and compared to commercially available agent Magnevist®. The latter had only around 2% of the injected doses in the blood left after 2 h, while the RBC-Gd-PLGA NPs were only removed to this level after around 48 h. The long blood circulation times of RBC coated particles was utilized for continuous detection of sodium levels in the bloodstream, which is of importance in detecting lithium side effects.[7a] The developed sensors could continuously measure sodium levels, which activated a fluorophore as response. This signal was analyzed via the recently developed technique “diffuse in vivo flow cytometry”, which can detect and quantify fluorescent signals in the bloodstream. The signal in the blood stream dropped to 0.2% of the initial value after 72 and 336 h for PEGylated version and the RBC membrane coated sensors, respectively.

Platelets have natural affinity for inflammatory sites. Consequently, using their cell membranes as coatings for nanoparticles has been considered. In contrast to current approaches for inflammatory targeting there is no need to rely on passive targeting due to the increased permeability of inflammatory tissue, or active targeting via ligands and external stimuli.[20] For example, Wang and co-workers used platelet membranes to coat PLGA NPs to target rheumatoid arthritis in a mice model, aiming to improve targeting due to the natural recruitment of platelets to rheumatoid arthritis, rather than relying on passive targeting via leaky vessels or adhesion of labor-intensive ligands now commonly used.[21] Platelet membrane coated PLGA NPs were not only outperforming the controls in targeting of rheumatoid arthritis tissue, but histological data confirmed a reduction in inflammatory markers. Platelets can also have affinity for tumor tissue. This aspect was exploited by coating Irinotecan-loaded gelatin nanogels with platelet membranes to target colon cancer.[7e] These platelet coated nanogels had the most significant tumor growth inhibition (66%) in mice compared to free Irinotecan (39%) and uncoated nanogels ($\approx 51\%$).

From a different perspective, Cheng and co-workers encapsulated cardiac stem/stromal cells secretome in PLGA to form nanocells and coated these with prostaglandin E2 (PGE$_2$) decorated platelet cell membranes for the treatment of heart ischemic/reperfusion injury.[22] The group has previously shown the homing ability of platelet membranes to ischemic cardiac tissue, while PGE$_2$ can interact with several receptors overexpressed in ischemic cardiac tissue, improving targeting and aiding regeneration.[23] Results from ex-vivo analysis of a mice showed a strong (ca. 15×) increase in fluorescence in the heart for the coated nanocells compared to the uncoated counterparts, as well as a 1.6× increase in viable myocardial tissue. In another approach, platelet cell membrane coated Gd PLGA NPs were used for imaging of the cardiovascular disease atherosclerosis.[24] These NPs were better adhering to the atherosclerosis disease site compared to PEG coated and RBC membrane coated PLGA NPs, and allowed for live MRI imaging of the atherosclerosis site in mice. A similar concept was used by Ge and co-workers, where PLGA core had the immunosuppressant rapamycin incorporated and was coated with platelet membranes to treat atherosclerosis (Figure 1bI).[25] The potential of targeted delivery was tested in mice. The examined atherosclerosis tissue exhibited a 5-fold increased fluorescence signal
when the platelet membrane coated particles were used compare to the uncoated control (Figure 1bII). Further, compared to a PBS control, the atherosclerosis plaque area decreased by 70% for rapamycin-loaded platelet coated NPs, while free rapamycin only had a 30% reduction (Figure 1bIII).

From a different perspective, the field of nano/micromotors has recently noticed the relevance of cell membrane coatings. The interaction of platelets with pathogens and toxins was combined with the mobility of a nanomotor for potential detoxification or pathogen treatment. The nanomotors had a palladium helical core that was coated with layers of Ni, Au and platelets membranes (Figure 1cI). The propulsion induced by applying a magnetic field was improved 2-fold for nanomotors with platelet cell membrane coating in blood (Figure 1cII). When nanomotors self-propelled through a suspension of MRSA252 bacteria, the fluorescent signal originating from the nanomotors revealed a 10-fold increase compared to passive nanomotors or platelet vesicles.

Figure 1. Non-nuclei containing cell sources. A) I) Collection of RBC membranes from mice and coating of RBC-shaped particles with the RBC membranes. II) Fluorescent signal in blood samples collected over a time span of 24 h after injection of uncoated (b) and RBC membrane coated particles (a). Reprinted with permission. Copyright 2018, American Chemical Society. B) I) Formation of platelet membrane coated rapamycin (RAP) loaded PLGA NPs (RAP-PNP). II) Optical analysis of aortas ex vivo after injection of PBS, uncoated NPs (RAP-NP) and RAP-PNP in mice. III) Assessment of atherosclerosis tissue after treatment of mice with PBS, free RAP, RAP-NP and RAP-PNP. Reprinted with permission. Copyright 2019, Elsevier. C I) Fabrication and coating of platelet membrane coated nanomotors. II) Mobility of uncoated and coated nanomotors in blood, with and without 1 h incubation before motion was induced. Reprinted with permission. Copyright 2018, Wiley.
4. Nuclei containing cells
The majority of cells have a nucleus, which contains their genetic information and allows for proliferation. The ability to divide means capacity to cultivating cells, which is the basic requirement for potential large-scale production. However, the additional cellular components makes the separation and purification of cell membranes more complex. A wide spectrum of nuclei containing cell are used as sources, i.e., white blood cells (WBC), cancerous cells and stem cells.

4.1. White blood cells (WBC)
WBCs are part of the immune system and have a function in inflammatory reactions and removal of foreign intruders. Therefore, WBCs can be related to a variety of diseases or infections, a property of interest for cell membrane coating for targeting these sites. The use of cell culture to obtain WBC and their expression of major histocompatibility complex, could make WBC membranes less biocompatible and compromise immunogenicity.[9b]

On the other hand, employing WBC membranes in tumor targeting is interesting due to their response to inflammation tissue caused by the tumor and the recruitment of some WBC by tumors to aid in tumor progression. Cai and co-workers sought to use WBC membrane coated PEG-PLGA co-polymer NPs with incorporated 4,4,4-(porphine-5,10,15,20-tetranyl)tetrakis(benzoic acid) photodynamic agent for a combined immune and photodynamic therapy.[27] The combination therapy was tested in mice resulting in 50% survival after 60 days, while uncoated NPs or single treatments showed no survival of the animals. The natural killer cell line NK-92 is of great interest due to its culture capability and lack of inhibitory receptors, increasing the cytotoxicity against maleficent cells. Membranes from NK-92 cells, were fused with liposomes to form NKsomes to target cancer, using doxorubicin as chemotherapeutical drug.[5a] Results from tumor inhibition experiments in mice showed that NKsomes inhibited tumor growth (∼79%) more than bare doxorubicin (∼64%). The use of WBCs was also explored for suppression of inflammatory response in rheumatoid arthritis via the use of neutrophil decoys.[28] PLGA NPs were coated with neutrophil membranes (neutrophil-NP), and the ability to bind two factors (IL-1β and TNF-α) linked to the disease was illustrated. The neutrophil-NPs seemed slightly better in retaining cartilage and reduction of joint swelling in mice compared to either IL-1β or TNF-α. A similar decoy strategy was utilized to treat human immunodeficiency virus (HIV) infection using PLGA NPs coated with CD4+ T-cell membranes, a type of WBC.[29] The virus naturally targets the CD4+ T-cell for replication, which results in the approach being relatively unaffected by mutations or strain variations of the virus. Results from binding tests with viral glycoproteins HIV-1 gp120 and HIV-1 gp120 showed that the virus could bind to CD4+ T-cell membrane coated NPs, similar as it would bind to CD4+ T-cells. In a sequential test, the ability to inhibit CD4+ T-cell death by binding of the virus with TNP was investigated. The viability of CD4+ T-cells increased with 30% when a mixture of gp120 proteins and CD4+ T-cell membrane coated NPs were used compared to a PBS control. WBCs can also be used as a nature-inspired solution for pathogen removal. In a different approach, macrophage cell membranes were used for coating of gold/silver nanocages for photothermal therapy against pathogens.[7c] Before harvesting the macrophages’ cell membranes, the macrophages were incubated for 3 h with the target pathogen to increase the amount of receptors for the specific pathogen on their plasma membranes. The anti-pathogen ability was tested by NPs incubation with Staphylococcus aureus and sequential NIR (5 min, 1 Wcm⁻¹) irradiation followed by the growth curve analysis of the surviving pathogens. Results showed that the NPs coated with the pre-incubated macrophages had half the amount of cell forming units compared to NPs coated with membranes from non-incubated macrophages. The interaction between cell membrane coated particles and cells is a key factor towards their successful performance. In this context, Yu and co-workers investigated receptor-ligand
interactions between cell membrane coated particles and lipid bilayers.\[^{8a}\] They used a 200 nm green fluorescent core that had a smaller 40 nm red fluorescent NP attached. These assemblies were coated with Jurkat T-cell membranes (Figure 2aI). The NPs were tracked on a supported lipid bilayer and information about receptor-ligand binding due to particle motion was obtained by following the motion of the fluorescent dyes. The analysis showed that the NP motion was restricted over time as more receptor-ligand binding events occurred (Figure 2aII).

Figure 2. Nuclei containing cell sources. A I) Formation of T-cell membrane camouflaged nanoprobes. II) Motions observed of cell membrane coated nanoprobes when interacting with a lipid bilayer. Reprinted with permission.\[^{8a}\] Copyright 2018, American Chemical Society. B) I) Formation of doxorubicin NPs with NIR active indocyanine green (ICG) and coating with HeLa cancer cell membranes. II) Release profile of doxorubicin from uncoated NPs and cell membrane coated NPs with and without NIR irradiation. Reprinted with permission.\[^{31}\] Copyright 2018, Elsevier. C) I) Coating of microparticles with gold and G422 cancer cell membranes to yield micromotors. II) Results from collision experiments with G422 cells and the attachment of the uncoated and cancer cell membrane coated micromotors. Reprinted with permission.\[^{5b}\] Copyright 2019, Wiley.

4.2. Cancerous cells
The use of various cancerous cell sources for cell membrane purification originates from their strong homologous targeting, making them interesting for different types of cancer treatment or tumor imaging.\[^{9a, 9e}\] A recent example in this context used cancer cell membranes from B16-F10 cells to coat mesoporous silica NPs (CMSN).\[^{30}\] They loaded the CMSN with glucose oxidase for starvation therapy, and PD-1 antibodies were simultaneously administered for immunotherapy. Accumulation of CMSN in mice showed strong tumor targeting, likely due to
homologous targeting and retention of CD-47 in the cell membrane coating. Further, only a combined treatment of CMSN together with PD-1 antibodies had a mouse survival rate of 20%, highlighting the potential of dual therapies. In a different dual therapy for cancer treatment relying on chemo and photothermal therapy, cancer cell membranes from HeLa cells were also employed.\textsuperscript{[31]} Doxorubicin NPs coated with the photothermal agent indocyanine green were co-extruded with cancer cell membranes resulting in vesicles containing one or more NPs in the core. (Figure 2bI). The photothermal properties were analyzed based on the heat generation after NIR irradiation (3 Wcm\textsuperscript{-2}, 5 min) of the NPs. The cancer cell membrane coating did not diminish the photothermal effect, and the NPs showed a burst release of doxorubicin after irradiation, likely due to the heat-induced rupturing of the HeLa cell membrane coating (Figure 2bII). In a different study, U87 glioma or human breast cancer cells membrane coated PLGA NPs (CCMF-PLGA-NPs) were used by Bhujwalla and co-workers for both inhibition of cancer cell migration towards fibroblasts as well as activation of the immune system.\textsuperscript{[32]} The CCMF-PLGA-NPs were used as decoys to interfere with cancer cell and fibroblast interactions as part of the cascade starting metastasis of the tumor. The metastasis inhibition was analyzed in mice injected with luciferase-expressing breast cancer cells (MDA-MD-231) and compared to a PBS control. The metastatic burden was around 0.2\% for mice treated with CCMF-PLGA-NPs compared to 4\% for the control.

Alternatively, the potential to use cancer cell membranes to create a cancer vaccine has been investigated, as the cancer cell membranes retain the antigens needed to activate the immune system as recently discussed in detail by Zeng and Pu.\textsuperscript{[33]} To this end, PLGA NPs were loaded with the adjuvant R837, coated with B16-OVA cancer cell membrane and decorated with mannose to improve dendritic cell uptake as a potential cancer vaccine.\textsuperscript{[34]} The NPs were tested \textit{in vivo} in a mice model and showed a two-fold reduction in tumor volume compared to a blank. However, when the NPs were combined with anti-PD-1, a drug for checkpoint blockade therapy, the tumor volume could be reduced a ten-fold compared to the blank, highlighting the synergistic effect.

The coating of NPs with cancer cell membranes is also interesting for imaging purposes again due to the homologous targeting of these CM-NPs. For example, MRI active superparamagnetic iron oxide NPs were encapsulated in polystyrene NPs and coated with the NIR active compound Chlorin e6 and cancer cell membranes from SMMC 7721 cells.\textsuperscript{[8b]} The particles had both, MRI and NIR-fluorescence imaging capabilities of the tumor site. After NIR irradiation (1 Wcm\textsuperscript{-2}, 10 min), the tumor weight was reduced to 100 mg after two weeks compared to >1000 mg for the PBS control. Furthermore, tumor cells show enhanced micro-RNA expression, which has gained interest as a potential biomarker for detection of cancer.\textsuperscript{[35]} Photoacoustic imaging is an interesting way of visualizing micro-RNA using specific photoacoustic agents. For example, mesoporous silica NPs were sequentially coated with a photoacoustic agent and MCF-7 cell membranes for tumor imaging.\textsuperscript{[36]} The signal from tumor bearing mice could be observed using photoacoustic imaging for up to 9 h.

From a different perspective, micromotors with a dual acoustic and bubble formation propulsion were coated with G422 cell membranes for targeting of tumor sites or immune activation (Figure 2cI).\textsuperscript{[5b]} The micromotors were propelled to collide with tumor cells and around 47\% were sticking to the cells (Figure 2cII). For comparison, micromotors without G422 cell membrane coating only attached in 12\% of the cases. In addition, mice on immune activation had higher increase of various immune factors (TNF-\(\alpha\), IL-12p40 and IFN-\(\gamma\)) when exposed to the G422 cell membrane coated micromotors compared to bare micromotors or cancer cell membrane vesicles.
4.3. Other cell types
In addition to WBC and cancerous cells, other nuclei containing cells were used for cell membrane coatings. These cells sources often had specific properties beneficial for the aimed purpose. For instance, cell membranes from tumor associated fibroblast have homologous targeting but without restriction to the tumor type. Semiconducting NPs made from a triblock copolymer consisting of PEG, poly(propylene glycol) and poly(cyclopentadithiophene-alt-benzothiadiazole) were used upon coating with cell membranes from these cell types and employed in phototherapy. These NP could eradicate the tumor nearly fully in mice, while 4T1 cancer cell membrane coated NPs resulted in a tumor weight of 0.3 g.

Mesenchymal stem cells (MSCs) have shown affinity for inflammatory and tumor sites and their cell membranes were therefore used for NP coating. Nie and co-workers used MSC membranes to coat doxorubicin-loaded PLGA NPs (PM-NP-Dox) to target tumor sites. Double accumulation of PM-NP-Dox in tumor site and around 40% increase in tumor inhibition compared to doxorubicin-loaded liposomes was found in mice. Limb ischemia, a disease where blood flow through the limb becomes insufficient or stops, has shown potential benefit from stem cell treatment due to targeting of the disease site via C-X-C chemokine receptor type 4 (CXCR4). However, instead of using the entire stem cell, Lee and co-workers employed MSC membranes that overexpressed the CXCR4 receptor to coat PLGA NPs loaded with vascular endothelial growth factor. Mice treated with these NPs had 83% lower full limb loss compared to untreated mice. In another effort, stem cell membrane coated NPs were used both to image and treat cancer using gene and photothermal therapy. Iron oxide NPs were coated with dopamine before adhesion of siRNA and coating with MSCs membranes. The improved tumor MRI imaging capabilities of the MSCs membrane coated NPs was confirmed in mice with DU145 tumors.

In another report, the CXCR4 receptors was overexpressed in neural stem cells (NSC) before the cell membranes were collected and used to coat PLGA NPs to target ischemic brain caused by a stroke. The ability to reach the ischemic tissue in the brain was investigated for bare NPs, NSC membrane coated NPs (with and without overexpressed CXCR4 receptor). Results from mice brain analysis showed a 2.7× and 2.1× increase in fluorescence originating from NSC CXCR4 overexpressed membrane coated NPs and NSC coated NPs, respectively, compared to uncoated NPs. Finally, NSC CXCR4 overexpressed membrane coated NPs were loaded with glyburide, an anti-stroke therapeutic. The infarct volumes decreased by 58% compared to a PBS control in mouse model.

In an alternative approach, Zhang and co-workers used gastric epithelial cell membrane coated NPs as potential oral treatment against Helicobacter pylori. Enhanced accumulation of fluorescent labeled NPs was observed after incubation with the pathogens in a PBS solution and sequential washing compared to PEGylated NPs due the inherent adhesion of the pathogen to epithelial cells. Despite incomplete bacteria removal in mice was found during the 4-week experiment, the number of colony forming units in a gram of mouse stomach tissue changed from $1.6 \times 10^5$ to $6.5 \times 10^3$ and $1.46 \times 10^2$ for PEG NP or cell membrane coated NPs, respectively. Further, cell membrane coated NPs were used to investigate the interactions of drug candidates with cell membranes. α1A-AR expressing HEK 293 cell membranes were used to coat carbon nanotubes with bound magnetic Fe3O4 for magnetic collection and subsequent analysis of the bound compounds. Two compounds, benzoylmesaconine and lappaconitine, were isolated from a mixture extracted from the herb Radix aconiti based on their interaction with the α1A-AR receptor on the cell membranes. These compounds were tested for their relaxing effect on muscles in a vascular ring assay, where a relaxation effect was seen for lappaconitine, but benzoylmesaconine only showed a weak signal.
5. Hybrid cell membrane coating particles

The thus far discussed examples rely on a single type of cell membranes to coat particles. However, combing cell membranes from different types of cells is an alternative approach that results in hybrid membrane coatings that exhibit duality of properties. It should be noted that combining natural cell membranes with lipids also creates a hybrid coating, but this is not the focus of this section. This aspect was recently discussed in more details in a review by Dai and coworkers.\[44\]

As outlined above, cancer cell membranes for coating were extensively used due to their targeting abilities. However, the maleficent origin makes them prone to recognition and removal by the immune system. On the other hand, particles coated with membranes originating from WBC are often well camouflaged. The combination of WBC membranes and HN12 cancer cell membranes was recently explored as coating for paclitaxel-loaded liposomes NPs.\[45\] These hybrid cell membrane coated NPs had a higher uptake in cancer cells than in macrophages, while coating with a single type of membrane only resulted in more uptake in macrophages compared to the cancer cells. Further, the tumor growth in mice exposed to the NPs with the hybrid membrane coating was almost inhibited while tumors doubled in size for the controls after 30 days. In a similar approach by Yang and co-workers, cancerous cell membranes from MCF-7 cells were fused with RBC membranes to find a balance between targeting and blood circulation times (Figure 3aI).\[46\] The retention of proteins after hybrid membrane coating was observed. Interestingly, the cell membranes did not mix before being extruded (Figure 3aII-III). Complete tumor ablation in mice could be achieved when NPs were coated with a 1:1 membrane protein weight ratio of RBC and MCF-7 cells. Photothermal therapy for cancer treatment was also considered for polypyrrole NPs coated with a combination of RBC membranes and platelet membranes.\[45\] This combination was chosen to create NPs with prolonged blood circulation times and an affinity for damaged blood vessels. The hypothesis posed was that after the initial photothermal therapy, damage to blood vessels occurred, which could assist guiding the NPs to the target tumor site. The hybrid coating outperformed both pristine RBC membrane coatings and platelet membrane coatings with a tumor mass of 0.140 g compared to 0.424 and 0.350 g, respectively. In an effort by Zhang and co-workers, a dual photothermal and chemo therapy as well as hybrid coating was utilized for cancer treatment.\[49\] The core was made from doxorubicin loaded copper sulfide NPs and the coating consisted of RBC and B16-F10 cancer cell membranes. Based on tumor accumulation studies in mice, NPs with the hybrid coating had the highest accumulation. The dual therapy could inhibit the tumor growth while hybrid NPs without irradiation showed limited inhibition, and lack of chemotherapy significantly reduced the tumor growth but without complete inhibition. Tumor-associated macrophages are of interest in cancer immunotherapy, as the two main sub-types M1 and M2 enhance and reduces inflammatory response, respectively. Therefore, depletion of M2 subtype could enhance immune response towards the tumor and could be used as a potential anti-cancer therapy. The Yang group explored this concept using BLZ-945-loaded dextran-graft-poly(histidine) NPs coated with RBC and 4T1 cancer cell membranes (DH@ECM) (Figure 3bI).\[50\] Dextran was able to target the M2 subtype over the M1 (3.5×), but the hybrid cell membrane coating blocked this interaction. The acidic environment in tumors was used to solve this challenge since the DH@ECM NPs would swell at low pH, rendering the cell membrane coating unable to cover the entire enlarged surface area (Figure 3bII). Analyses from tumor inhibition experiments in mice showed the best results for DH@ECM NPs (64.5%), while uncoated NPs (43.6%) and free drug (21.3%) showed reduced inhibition (Figure 3bIII).

The use of RBC membranes and platelet membranes coated particles for targeting pore forming toxins and pathogens, respectively, was also combined with nanomotors to benefit from their
enhanced locomotion$^{[51]}$. The mobile hybrid coated nanomotors outperformed stationary nanomotors and cell membrane ghost particles by 3.4-fold higher pathogen removal and 1.5- to 4-fold increase in toxin elimination. Finally, these two properties were tested sequentially using the same nanomotors, which resulted in a 3.1- and 2.2-fold reduction of pathogens and toxins, respectively, compared to the control.

Figure 3. Hybrid cell membrane coated particles. A) I) Coating of melanin NP with RBC and MCF-7 cancerous cell membranes. II) Confocal laser scanning microscopy images of individual RBC membranes vesicles (top-left), MCF-7 cell membrane vesicles (top-right), a mixture of these vesicles (bottom-left) and hybrid cell membrane vesicles (bottom-right). III) Sodium dodecyl sulfate-page polyacrylamide gel electrophoresis and Western blot results of individual and hybrid cell membrane vesicles and after coating on melanin NPs. Reprinted with permission.$^{[46]}$ Copyright 2019, Elsevier. B) I) Formation of hybrid RBC and 4T1 cancer cell membrane coated dextran/histidine NPs loaded with BLZ-945. II) Drug release profile of hybrid cell membrane coated NPs (DN@ECm) and uncoated NPs (DN) in various pH. III)
Inhibition of tumor growth in mice for the free drug, DN and DN@ECm. Reprinted with permission.[50] Copyright 2020, Elsevier.

6. Conclusion and outlook
The use of cell membrane as coating for particle is a concept, which emerged in the last decade to camouflage synthetic nanomaterial, typically for biomedical applications. The proteins retained in the cell membranes can aid in increasing blood circulation times and effective targeting of various disease sites among others. In general, cell sources for purifying cell membranes should be easily available in large numbers in order to transfer an inherent property of the cells to a synthetic material. RBC aims at prolonged blood circulation times while platelets focus on targeting of injured blood vessels, cancer, arteriosclerosis or bacteria. The envisioned applications are diverse from sensing over imaging and targeting of diseases either passive or via platelet affinity. The use of nuclei containing cells is largely focusing on tumor targeting/imaging due to cancerous cells’ homologous targeting ability. Further, inflammatory and pathogen targeting with WBC membrane coated particles was explored. Stem cells, tumor associated fibroblast, and gastric epithelial cells were used for the treatment of ischemic tissue, tumors or bacterial infections, respectively. Finally, the ability to fine-tune the control over the cell membrane coatings, combinations of different cell sources were employed, resulting in more balanced performance between, e.g., targeting and blood circulation times.

Although different types of cell sources, core types and anticipated applications are being explored, many aspects need to be elucidated and understood in more detail. The results obtained to date are promising and wider applications of this concept can be anticipated in the future when a variety of limitations are overcome. For instance, long-term safety and stability will need to be determined. Furthermore, the sourcing and expanding of cells for cell membrane harvesting is likely to face challenges when larger scale production is envisioned. RBC and platelets cannot be cultivated and need donor blood. On the other hand, preservation of phenotype for cultured cells might be of key importance for their anticipated function. The largest challenge today is likely the cell membrane purification efficiency. Novel concepts that do not only rely on separation via centrifugation will need to be identified and combined with effective (nano)particle coating and storage procedures. Further, while the focus today is on intravenous injectable nanoformulations, often for cancer therapy, cell membranes could be found useful for coating of implantable biomaterials or engineered materials that are supposed to interface with (human) cells and tissue. In recent examples in this context, cell membrane vesicles are used as crosslinking agents for hydrogels.[52] In addition, RBC membranes as coating for glucose sensors were explored.[53]

Over the last years, the use of cell membranes as coating has advanced towards applications in sensing, potential treatments of a variety of medical conditions, the use of increasing diverse types of cell sources and the increased consideration of hybrid cell membranes on the way to become a clinically relevant concept. In other words, cell membranes are a core part in living cells, but they are also powerful building block that can be used for nature-inspired engineering of materials for biomedical applications.
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References


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Cell membranes that are purified from mammalian cells can be used as coating of particles to equip them with unique cell-lookalike properties. Currently, these cell membrane coatings are typically envisioned to camouflage different types and shapes of nanomaterials with the aim to improve the targeting ability and blood circulation times of nanoformulations.

**Cell Membrane Vesicles**

J. M. Spanjers, B. Städler

Cell Membrane Coated Particles